weight to be around 200 kDa. The two polypeptides of δ crystallin, $\delta1$ and $\delta2$, are known to be two distinct gene products which share a 91% homology (Nickerson *et al* 1986; Wawrousek and Piatigorsky 1987). We work here largely with $\delta1$-crystallin. Our analysis of the sequence has shown that δ-crystallin has a 13-residue-long non-helicogenic region (residues 318–330) rich in anionic charged amino acids, flanked on either side by helicogenic residues (300–317 and 331–350) that can fold into amphipathic α-helix. This region is typical of the 'E helix–loop–F helix' motif of calcium binding proteins, and has been suggested as the binding site for calcium in δ-crystallin (Sharma *et al* 1989).

We have further noted that calcium binding to δ-crystallin perturbs the vibronic fine structure of the aromatic side chains in δ-crystallin. The aromatic CD spectrum, in the absence of calcium, shows a well-defined structure (figure 1). The minimum at 292 nm is attributed to the ^1L_b band of Trp which also has a contribution at 268 nm, while the three prominent band minima at 274, 278 and 285 nm are assigned as the vibronic bands of the ^1L_b bands of the Tyr chromophore (Strickland 1974).

The far UV CD spectrum of δ-crystallin (figure 1) is characteristic of high α-helical content, in agreement with earlier studies on both δ-crystallin in the lens *in situ* by Raman spectroscopy (Kuck *et al* 1976; Yu *et al* 1977) and in solutions of purified δ-crystallin (Piatigorsky *et al* 1977; Williams *et al* 1982). The α-helical content of embryonic chicken δ-crystallin has been found to be as high as 82% (Horwitz and Piatigorsky 1980).

The fluorescence spectrum of δ-crystallin excited at 280 nm shows a shoulder at 307 nm and a doublet at 315 and 325 nm (figure 2a). Since both Tyr and Trp are excited at this wavelength, the assignment of the emission bands poses a problem. Upon excitation at 295 nm (or at 300 nm), the protein shows a highly blue-shifted fluorescence doublet at 315 and 325 nm, but the shoulder at 307 nm is absent. As the absorption of Tyr at 295 and 300 nm is negligible, the emission doublet is assignable

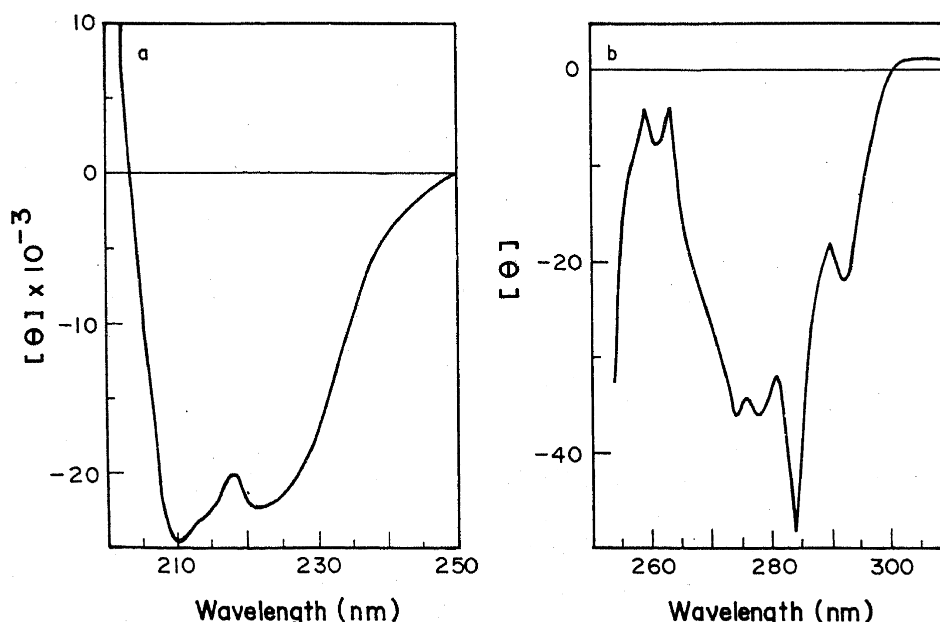

Figure 1. Circular dichroism spectra of δ-crystallin: (**a**) peptide absorption region; (**b**) aromatic absorption region. Protein concentration around 0·8 mg/ml in *p*H 7·0 phosphate buffer. Ellipticity in units of deg.cm^2.dmol^{-1}.